DALHOUSIE

2016 Protocols

Illumina MiSeq Metgenomic Sequencing Analysis

Adapted from:
Reference: Metagenomic standard operating procedure in https://github.com/mlangill/microbiome_helper/wiki/Metagenomic-standard-operating-procedure

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Purpose:
To perform functional analysis on Illumina MiSeq shotgun metagenomics

Requirements:

- Command line terminal (e.g. Mac OS Terminal, VirtualBox)
- FastQC
- PEAR
- Bowtie2
- Human pre-indexed database
- DIAMOND (> v.0.7.0)
- MetaPhlAn2
- HUMAnN

Procedure:
(Reference: Metagenomic standard operating procedure in https://github.com/mlangill/microbiome_helper/wiki/Metagenomic-standard-operating-procedure: terminal commands can also be found here.)

1. (Optional) Run FastQC to allow manual inspection of the quality of sequences.
2. Stich paired end reads together.
3. Run Bowtie2 to screen out contaminant sequences, here we are screening out reads that map to the human or PhiX genomes.
4. Run Trimmomatic to trim off bases under specified quality values and to discard reads under a certain length after trimming (running FastQC on the screened reads is helpful for choosing these parameters).
5. Run MetaPhlAn2 for taxonomic composition.
6. Convert from MetaPhlAn to STAMP profile file.
7. Run pre-HUMAnN (DIAMOND search).
8. Run HUMAnN (link files to HUMAnN "input" directory and then run HUMAnN with scons command).
9. Convert HUMAnN output to STAMP format.